

# Real-Time PCR Panel/ Roundtable Discussion:

## Real-Time PCR in a Core Lab Setting

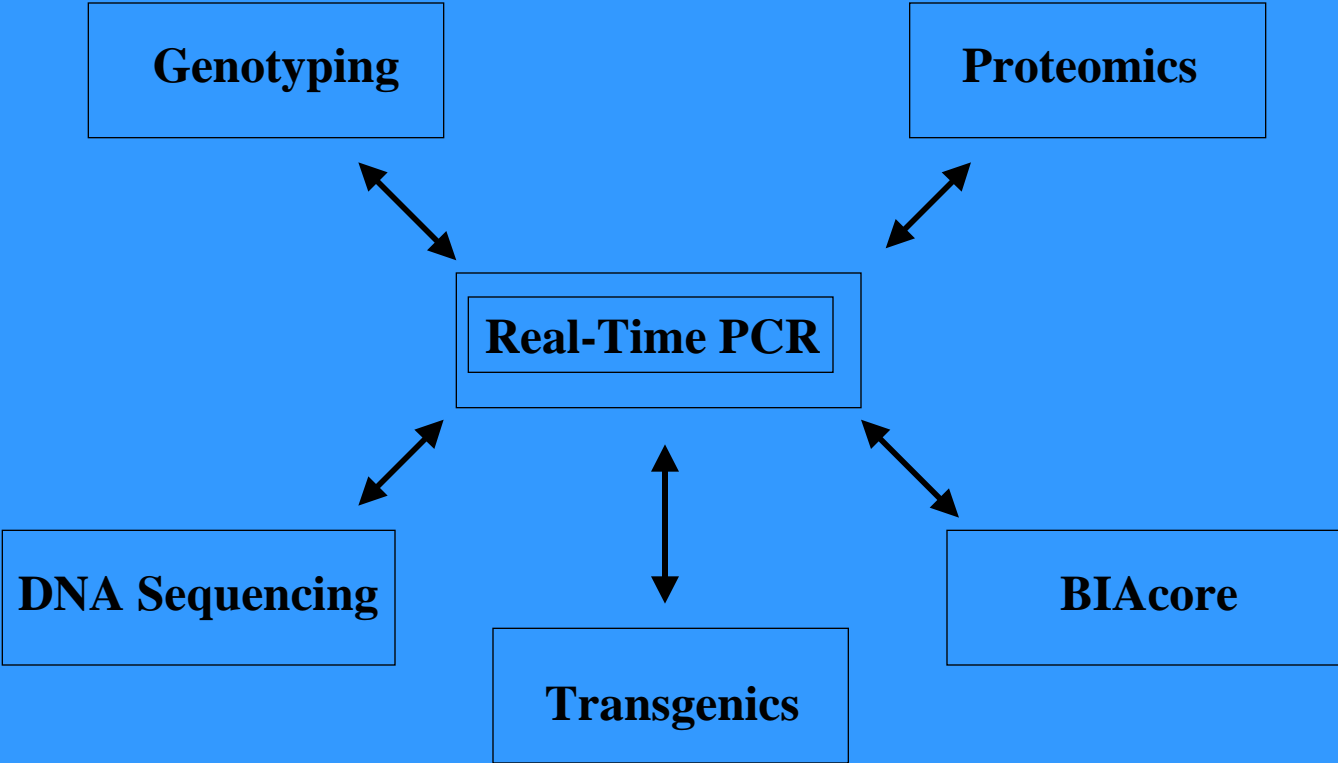
- Panel Participants
  - Chair: Scottie Adams, Trudeau Institute
  - John Hawes, Indiana University School of Medicine
  - Deb Grove, Pennsylvania State University
  - Brian Holloway, Centers for Disease Control
  - Tim Hunter, University of Vermont
  - Kevin Knudtson, University of Iowa
  - Greg Shipley, Univ. of Texas-Houston Medical School

# Real-Time PCR in a Core Lab Setting: Factors to Consider

**John Hawes**

**Indiana University School of  
Medicine**

# Real-Time PCR in a Core Lab Setting



# **Shared Instrument or Service?**

**Depends on the Setting and the Applications**

# **One Size Does Not Fit All**

**Different Applications**

**Different Instruments**

**Different Throughput**

**Different Reagents**

**Different Budgets**

# Different Applications

- **Gene Expression**
- **SNP Analysis / Mutation Detection**
- **Viral Titer**
- **Bacterial Species Identification**
- **Chromosomal Deletions / Duplications**

## Different Instruments

### What are the differences?

- **Fluorescent or Optical Detection**
- **96 wells / fewer wells / capillaries**
- **Different Chemistries**
- **Different Throughput**

# **Some Good Things to Have**

**Extra Tools (plate holders, cap sealers, etc.)**

**Extra Plates and Caps (hidden away for emergencies)**

**Another Freezer / Refrigerator**

**A Desk-Top Centrifuge with a Plate Rotor  
(Better than a Salad Spinner)**

**Extra Copies of Manuals**

**Extra Copies of Software (legally of course)**

## Some Good Practices

Offer Advice for Experimental Design and Sample Prep

Mandatory Training Sessions.

Make a “Sign-up Sheet” and Enforce it.

Regular Training Lectures.

Keep a Stock of Training Reagents.

Get Users Together to Share Experiences and Ideas.

## Fee Schedule?

**Depends on the Setting and the Applications**

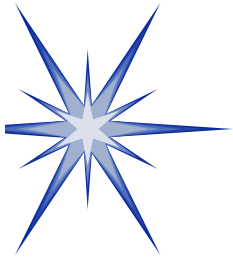
## Where can you learn more?

ABRF Electronic Bulletin Board

Real-Time PCR List Serve:

<http://groups.yahoo.com/group/qpcrlistserver/>

# **Examples of Real-Time PCR Core Facilities**



# Real-Time PCR Roundtable

Tim Hunter: University of Vermont

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INSTRUMENT(S):	7700 Sequence Detection System
SERVICES OFFERED:	BADA, SNP, Relative and Absolute Quantitation, Enzyme Kinetics
LEVEL OF SERVICE:	Comprehensive (probe, primer design, RNA, DNase, RT-PCR protocols, output analysis) Depends what level they buy into. <b>Three levels of service offered:</b> Full, Self, 1/2 hr. rental
ASSAY DEVELOPMENT:	SNP (BADA)-run first set in real-time to view multicomponent Relative Quantitation-serially dilute sample and run target and housekeeping gene to check PCR efficiencies. Plot $\Delta C_t$ vs. Sample log input. If slope is $<0.1$ --perform Comp. Ct Method. If slope is $>.1$ , suggest Relative or Absolute quantitation.
One step vs. Two-step:	User defined. One-step preferred
Charges:	Full: \$5.50/well (Facility provides reagents and labor) Self: \$4.00/well (Facility provides reagents and consumables, no labor) 1/2 hr. rental: \$15.00 ( support only)

**TaqMan ORDER FORM**  
**VERMONT CANCER CENTER DNA ANALYSIS FACILITY**

Date ordered \_\_\_\_\_ Date needed \_\_\_\_\_  
Ordered by \_\_\_\_\_ Phone # \_\_\_\_\_  
Investigator \_\_\_\_\_ Budget # \_\_\_\_\_  
e-mail address \_\_\_\_\_ Budget End Date: \_\_\_\_\_

Target: \_\_\_\_\_ Probe/Primer conc: default \_\_\_\_\_ or  
optimized \_\_\_\_\_ Dye \_\_\_\_\_ Quencher: \_\_\_\_\_  
Target: \_\_\_\_\_ Probe/Primer conc: default \_\_\_\_\_ or  
optimized \_\_\_\_\_ Dye \_\_\_\_\_ Quencher: \_\_\_\_\_  
Target: \_\_\_\_\_ Probe/Primer conc: default \_\_\_\_\_ or  
optimized \_\_\_\_\_ Dye \_\_\_\_\_ Quencher: \_\_\_\_\_  
Target: \_\_\_\_\_ Probe/Primer conc: default \_\_\_\_\_ or  
optimized \_\_\_\_\_ Dye \_\_\_\_\_ Quencher: \_\_\_\_\_  
Endogen. control: \_\_\_\_\_ Probe/Primer conc: default \_\_\_\_\_ or  
optimized \_\_\_\_\_ Dye \_\_\_\_\_ Quencher: \_\_\_\_\_

# Samples: \_\_\_\_\_

Triplicates:                    yes \_\_\_\_\_ no \_\_\_\_\_

Duplicates:                yes \_\_\_\_\_ no \_\_\_\_\_

Multiplex:                yes \_\_\_\_\_ no \_\_\_\_\_

Standard curve:        yes \_\_\_\_\_ no \_\_\_\_\_ Sample name \_\_\_\_\_

Comp. Ct Method:        yes \_\_\_\_\_ no \_\_\_\_\_



# Real-Time PCR Services Trudeau Institute

- **Core provides**
  - **Training (no charge)**
  - **Reagents (at cost - approx. \$30/100 Rxns)**
  - **Primer/Probe and Assay Design (\$100/assay)**
  - **Analysis Assistance (no charge)**
  - **Equipment Maintenance and Support (no charge)**
- **User**
  - **Performs Assays (Charge = \$20/run)**

New users can obtain information about “Taqman” procedures and reagents from the MBCF webpage

Trudeau Institute  
Molecular Biology Core Facility  
**Taqman Reagents**

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INTRODUCTION:

This webpage order form can be used to order Taqman supplies and reagents and to obtain Taqman information.

- Taqman Assays are typically done by the user.
- There is a \$20/run chargeback to cover the cost of a service contract, maintenance, etc.
- The MBCF will provide training, supplies and reagents to get you started.
- The MBCF has designed and validated (with the generous help of Steve Smiley, Jonathon Preall, Frank Szaba, Nancy Lepak and Yu-jin Jung) a variety of [Primer/Probe sets](#) for quantitating mouse genes. These reagents are available from the MBCF for \$30/100 reactions.
- The MBCF will design any [new prime probe sets](#) that you require.
- Applied Biosystems has a number of [mouse PDAR](#) (Pre Developed Assay Reagent) Kits available. These are available on an immediate basis, but the initial outlay of cash is substantial, \$800 per 400 reactions and many of their kits also amplify genomic DNA.
- The Taqman machine can also be used to quantitate very small quantities (0.01 - 1.0 ng/ul) of [RNA](#).

HOW TO ORDER TAQMAN REAGENTS/SUPPLIES:

- Please SELECT the proper choices below.
- Submit the form to the MBCF by clicking on the SUBMIT button at the bottom of the page. You should receive an e-mail confirmation of the receipt of your order within 3 hours. If this does not occur, please contact Scottie at Ext. 115 or Tim at Ext. 181.
- There is a box at the bottom of the page for additional information or comments. IF YOUR NAME, etc. IS NOT ONE OF THE CHOICES, use Other and fill in the information in the Additional Information/Comments box. The information will be added to the webform for your next order.

Thank you for your cooperation.

A web based ordering system is used. The user fills out the form and the order is emailed to the core

NAME:  alphabetically by first name

LABORATORY:  alphabetically by last name

TELEPHONE EXTENSION:

CHARGE TO GRANT #:

REAGENT/SUPPLY/AMOUNT REQUESTED:

PLEASE fill in the number of each reagent/supply that you need.

2X Mastermix (400 Reactions/aliquot)

Tubes (100 Tubes/Pkg)

Lids (96 Tubes/Pkg)

B2microglobulin (100 Reactions/Aliquot) **NEW**

CD40L (100 Reactions/Aliquot)

ESAT-6 (100 Reactions/Aliquot) **NEW**

Flu A (100 Reactions/Aliquot)

Fgl-2 (100 Reactions/Aliquot)

GAPDH (100 Reactions/Aliquot)

GATA-3 (100 Reactions/Aliquot)

IFN  $\gamma$  (100 Reactions/Aliquot)

IL-1b (100 Reactions/Aliquot)

IL-2 (100 Reactions/Aliquot)

IL-4 (100 Reactions/Aliquot)

IL-10 (100 Reactions/Aliquot)

The reagents can be viewed to determine the status of new primer/probe sets under development.

"IN HOUSE" TAQMAN PRIMER/PROBE SETS			
Gene	Status	Genomic Amplification	Specificity Verified by DNA Sequencing
$\beta$ 2m	Ready to use	NO	NO
Ecl-3	In testing	NO	NO
CD40L	Ready to use	NO	YES
CCR3	In progress	NO	NO
CCR5	In progress	NO	NO
CCR7	Ready to use	NO	NO
CXCR3	In progress	NO	NO
CAPDH	Ready to use	YES	NO
GATA-3	Ready to use	NO	YES
GUS	Ready to use	YES	NO
HRT	Ready to use	YES	YES
FM $\beta$	Ready to use	NO	YES
L-1b	Ready to use	NO	NO
L-2	Ready to use	NO	NO
L-4	Ready to use	NO	YES
L-5	In Progress	NO	NO
L-10	Ready to use	NO	YES
L12 p35	Ready to use	NO	YES
L12 p40	Ready to use	NO	YES
NOS2	Ready to use	NO	YES
P-10	Ready to use	NO	NO
MCP-1	Ready to use	NO	YES
MCP-2	In Progress	NO	YES

# REAL-TIME PCR SERVICES AT THE UNIVERSITY OF IOWA

- Core provides

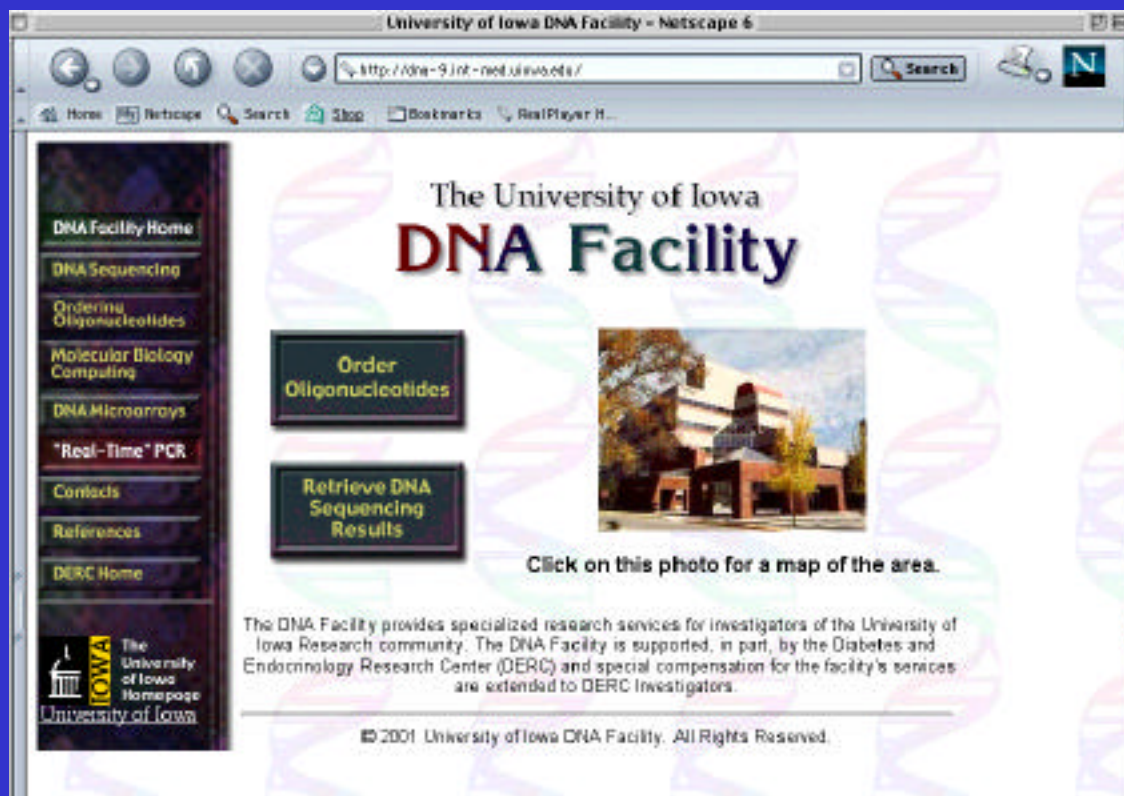
- Training and advice (no charge)
- Access to primer and probe design software and advice on its use (no charge)
- Analysis assistance (no charge)
- Access to the instrument (\$25/run)\*

- User's responsibilities

- Obtain necessary reagents and consumables
- Set up their own reactions--provide samples in a form that is ready to run on the instrument

\*Fee is designed to cover the yearly service contract and the odd consumable necessary to keep the machine operational

**Investigators are asked to first visit our web site to obtain introductory information about real-time PCR**



**Web site also contains information on how to use our service**

University of Iowa DNA Facility - "Real-Time" PCR - Netscape 6

http://dna-9.leaf.ned.iowa.edu/realtime.htm

Home Messages Search Shop Bookmarks RealPlayer H...

## "Real-Time" or Kinetic PCR

- [A. Real-Time Chemistry](#)
- [B. Instrumentation](#)
- [C. Real-Time PCR Quantitation](#)
- [D. Primer and Probe Design](#)
- [E. Thermal Cycling Parameters](#)
- [F. Sample Preparation](#)
- [G. Data and Analysis](#)
- [H. Supplemental Information](#)

The DNA Facility houses the "real-time" or kinetic PCR instrument, the Applied Biosystems Model 7700 sequence detection system (the TaqMan instrument). The polymerase chain reaction (PCR) has revolutionized the detection of DNA and RNA. As little as a single copy of a particular sequence can be specifically amplified and detected. Theoretically, there is a quantitative relationship between amount of starting target sequence and amount of PCR product at any given cycle. In practice, though, it is a common experience for replicate reactions to yield different amounts of PCR product. The development of real-time quantitative PCR has eliminated the variability traditionally associated with quantitative PCR, thus allowing the routine and reliable quantification of PCR products. This instrument, therefore, now provides investigators with the ability to perform very sensitive, accurate, and reproducible measurements of levels of gene expression. In addition, this instrument can be used in other applications such as measuring viral load, performing allelic discrimination studies, and optimizing PCR conditions.

The U of I  
DNA Facility

- [DNA Facility Home](#)
- [DNA Sequencing](#)
- [Ordering Oligonucleotides](#)
- [Molecular Biology Computing](#)
- [DNA Microarrays](#)
- ["Real-Time" PCR](#)
- [Contacts](#)
- [References](#)
- [DERC Home](#)

The University of Iowa  
Homepage  
University of Iowa

- Custom sample sheet created for each user
  - Provides information about the chemistry used in their reactions to help us set up the instrument appropriately
  - Provides information needed to bill their account
- User brings this sheet along with their samples and the core sets up and runs the instrument

**Request for Realtime PCR** **University of Iowa  
DNA FACILITY**

**Account ID:** 8100

**Account Principal Investigator:**  
**Kevin Knudtson**  
 Internal Medicine  
 3001 EMRB

**Realtime PCR Requested by:**  
 Name: \_\_\_\_\_  
 Phone: \_\_\_\_\_

U of I Master File Key

Emp ID	Fund	Org	Dept	Subdept	Grants Program	Inst. Acct	Org Acct	Dept Acct	PI	Cost Ctr
10	288	11	3000	18200	0000000	8218	000	00008	11	7513

**A. Project Name:** \_\_\_\_\_ **B. Probe Type:** \_\_\_\_\_  
(omit to generate file name)  TaqMan  Molecular Beacon  None

**C. Reaction Kit Source:**  PE Biosystems  Life Technologies  Stratagene  Wampersol (Other)

**D. Reagent Labels:**

<b>Reference_Dye</b>	<b>Reporter_Signal</b>	<b>Quencher_Dye</b>	<b>Interspersing_Dye</b>
<input type="checkbox"/> FRET	<input type="checkbox"/> 4-FAM	<input type="checkbox"/> FAM/MA	<input type="checkbox"/> SYBR Green
<input type="checkbox"/> None	<input type="checkbox"/> VIC	<input type="checkbox"/> DABCYL	<input type="checkbox"/> Other _____
<input type="checkbox"/> Other _____	<input type="checkbox"/> HEX	<input type="checkbox"/> Black Hole	
	<input type="checkbox"/> JOE	<input type="checkbox"/> Other _____	
	<input type="checkbox"/> TET		
	<input type="checkbox"/> Other _____		

**E. Reaction Conditions:** Use the blanks to change the default conditions, if necessary.  
Note: If you have already performed the RT step, then select PCR or Other

	<b>Stage 1</b>	<b>Stage 2</b>	<b>Stage 3</b>
	1   _____   Cycle	1   _____   Cycle	#   _____   Cycle
<input type="checkbox"/> PCR	58' _____ °C, 3 _____ min	95' _____ °C, 30 _____ min	95' _____ °C, 0:15 _____ min 60' _____ °C, 1 _____ min
<input type="checkbox"/> RT-PCR	48' _____ °C, 30 _____ min	95' _____ °C, 15 _____ min	95' _____ °C, 0:15 _____ min 60' _____ °C, 1 _____ min
<input type="checkbox"/> Other	_____ °C, _____ min	_____ °C, _____ min	_____ °C, _____ min _____ °C, _____ min _____ °C, _____ min

**F. Reaction Volume:** \_\_\_\_\_ µl **G. Are you multiplexing?**  Yes  No

DNA Facility: 301 Corbett Medical Research Bldg | Diagnostics and Epidemiology Research Center, College of Medicine | Phone: (319) 335-7058 | Fax: (319) 335-6737

**Submit Realtime PCR requests to room 321 EMRB**



## The Life Sciences Consortium

The Nucleic Acid Facility, Director Deborah S. Grove, Ph.D.

An ABI 7700 Sequence Detection System is available for Quantitative Real-Time PCR. The NAF provides QRT-PCR starting with RNA or DNA.

- \$4.50 a reaction starting with RNA (DNase step included) and \$3.60 starting with DNA. All reactions are set up in facility. Prices are higher for non-University customers.
- \$15 per plate run if set up yourself.
- Primer/Probe Design free for customers.
- Internal Housekeeping Genes available include 18S, GAPDH for a variety of species, and Ribophosphoprotein (36B4), rodent and human.
- Turnaround time is 48 hrs to 5 days depending on number of genes requested and number of samples already in the queue.
- See <http://www.lsc.psu.edu/stf/naf/Quantitative.html> for more information.